

# Variations in serum transforming growth factor- $\beta$ 1 levels with gender, age and lifestyle factors of healthy Japanese adults

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**Abstract.** Elevated serum or plasma Transforming Growth Factor- $\beta$ 1 (TGF- $\beta$ 1) levels have been linked to cancer and other diseases in numerous studies; however, very few studies have reported an association between circulating TGF- $\beta$ 1 and lifestyle factors in healthy people. We examined the association between serum TGF- $\beta$ 1 levels and gender, age, body mass index (BMI), smoking, and drinking in a large population-based cohort study (N = 9,142). Serum TGF- $\beta$ 1 levels were detected by the Quantikine enzyme-linked immunoassay kit (R&D Systems). The data indicated highly significant ( $p < 0.0001$ ) difference in serum TGF- $\beta$ 1 levels between men (mean value:  $37.6 \pm 0.12$  ng/mL, N = 4888) and women (mean value:  $35.1 \pm 0.12$  ng/mL, N = 4254). Serum TGF- $\beta$ 1 levels decreased with age (trend  $p < 0.0001$ ) and were positively associated with obesity (trend  $p < 0.0001$ ) in both men and women. We observed a significant trend with increased serum TGF- $\beta$ 1 levels corresponding to increased amount of tobacco and alcohol consumption in men (trend  $p < 0.0001$ ). These findings suggest that serum TGF- $\beta$ 1 levels appear to be modulated by gender, age and lifestyle factors such as obesity, cigarette smoking, and alcohol drinking in healthy Japanese adults.

Keywords: Serum TGF- $\beta$ 1, gender, age, smoking, obesity

## 1. Introduction

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is polypeptides that regulate several cellular functions, including cell growth, differentiation, motility, and extra cellular

matrix production [1]. Recent studies have also underscored the essential role of TGF- $\beta$  in maintenance of immune homeostasis [2]. TGF- $\beta$  regulates immune cells that are involved in both innate and adaptive immune response, exerting its immunosuppressive effects through inhibiting the function of inflammatory cells and promoting the function of regulatory T cells [3].

TGF- $\beta$  has three isoforms, of which TGF- $\beta$ 1 is the most abundant and universally expressed isoform. Circulating levels of TGF- $\beta$ 1 can be measured and have

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shown a potential usefulness as a prognostic marker for human diseases. High serum levels of TGF- $\beta$  1 have been found in patients with invasive prostate, breast, or colorectal cancer as compared with healthy control subjects [1,4–6]. Furthermore, the overexpression of TGF- $\beta$ 1 in cancer tissues and higher circulating levels significantly correlate with tumor progression, metastasis, angiogenesis and poor prognostic outcome [7–9].

Previous studies have shown a considerable variation in plasma TGF- $\beta$ 1 levels among healthy individuals, with the reported values greater than 100-fold range [10]. Besides, very few studies have examined the relationship between circulating TGF- $\beta$ 1 levels and lifestyle factors in healthy individuals. The aim of this study is to assess variations in serum TGF- $\beta$ 1 levels with gender, age and lifestyle factors among middle-aged and elderly Japanese individuals, the finding of which may be applied to potentially evaluate cancer risk in Japanese population.

## 2. Methods

The study population included healthy control subjects in a nested case-control study within a large cohort study, Japanese Collaborative Cohort Study for Evaluation of Cancer Risk (the JACC study). Details of the JACC Study have been described previously [12]. Briefly, the aim was to evaluate the association between lifestyle factors and cancer risk in middle-aged and elderly Japanese. Between 1988 and 1990, 110,792 subjects aged 40–79 years were recruited from 45 areas throughout Japan and subsequently followed up for mortality. Baseline data were collected through a self-administered questionnaire including information on demographic characteristics, medical history, and lifestyle factors such as cigarette smoking, alcohol drinking, and dietary habits. In addition, 35% of the cohort participants donated blood samples upon our request, and the sera were kept at  $-80^{\circ}\text{C}$  until analyses.

In the present study, we evaluated the association of serum TGF- $\beta$ 1 levels with age and selected lifestyle factors such as smoking, drinking and obesity in 9142 control subjects (4888 men and 4254 women) who came from a nested case-control study within the JACC Study. Those with missing data on TGF- $\beta$ 1 levels and each lifestyle factor were excluded.

Detailed information on lifestyle factors was collected using a self-administered questionnaire. For cigarette smoking, subjects were asked to describe their smoking status (current, former, or never) and

to respond to smoking-related questions including age at which smoking started, the number of cigarettes smoked per day, and years of smoking. In the section of alcohol drinking, we first asked the subjects to report their drinking status (nondrinker, ex-drinker, or current drinker). Data on the frequency of consumption, the type of beverages and the average amount of consumption on one occasion were further collected for ex-drinkers or current drinkers. Body weight and height were self-reported, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Informed consent was obtained mainly by having the subjects sign on the cover of the questionnaire; however, the community leaders in some study areas gave the consent for participating in the study on representative of the local residents. The JACC Study was approved by the Ethics Committee of Nagoya University School of Medicine.

### 2.1. Biochemical assay of sera

Serum TGF- $\beta$ 1 levels were measured by a quantitative sandwich enzyme immunoassay technique using a Quantikine human TGF- $\beta$ 1 kit, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). For serum preparation [11], the time of clotting is 30 minutes at room temperature. Then samples were centrifuged for 10 minutes at approximately 1000  $\times$  g after incubation. All samples were assayed at a single laboratory (SRL Inc., Hachioji). The intra-assay coefficient of variation for quality control samples ranged from 2.67 to 6.79% (13).

### 2.2. Statistical analysis

Since serum TGF- $\beta$ 1 levels seemed normally distributed for both men and women, we used raw measurement data in all analyses. Age- and area-adjusted least square means of serum TGF- $\beta$ 1 levels across categories of BMI, smoking and drinking were estimated using analysis of covariance methods. Analysis of covariance methods were also used to test for trends in TGF- $\beta$ 1 levels across each category. Statistical analyses were conducted using SAS Release 9.1 (SAS Institute, Cary, NC).

Table 1  
Mean values of serum TGF- $\beta$ 1\* levels in subcohorts of healthy Japanese adults classified by selected lifestyle factors

Category	Men (N = 4888)				Women (N = 4254)			
	N	TGF- $\beta$ 1	SE***	Trend	N	TGF- $\beta$ 1	SE***	Trend
Gender	Entire cohort (N = 9142)	4888	37.6	0.12	4254	35.1	0.12	$P < 0.0001$
Age (Yr)	40–49	282	40.6	0.52	397	36.9	0.46	Negative
	50–59	1104	38.7	0.27	960	35.7	0.35	correlation
	60–69	2585	36.9	0.21	1849	35.0	0.29	( $p < 0.0001$ )
	70–79	917	34.2	0.30	1048	33.4	0.34	
BMI** (kg/m <sup>2</sup> )	< 18.5	272	36.3	0.53	286	32.8	0.51	Positive
	18.5–24.9	3779	37.5	0.21	2947	35.3	0.28	correlation
	$\geq 25.0$	837	38.4	0.32	1021	36.2	0.34	( $p < 0.0001$ )
Cigarette smoking	Former and never	2468	37.4	0.23	4133	35.4	0.28	No
	Current, 1–19 cigarettes/day	902	37.3	0.33	85	35.2	0.84	correlation
	Current, $\geq 20$ cigarettes/day	1501	38.1	0.27	29	36.1	1.41	( $p = 0.77$ )
Alcohol drinking	Former and never	1276	37.0	0.30	3421	35.5	0.29	No
	Current, < 23 g/day	673	37.5	0.37	413	35.1	0.45	correlation
	Current, $\geq 23$ g/day	2050	38.2	0.27	68	35.6	0.95	( $p = 0.28$ )

\*TGF- $\beta$ 1 (ng/mL), Transforming growth factor- $\beta$ 1.

\*\*BMI, body mass index.

\*\*\*SE, standard error of the means.

### 3. Results

The summary of data for the entire population-based cohort study (N = 9142) is presented in Table 1. Mean values of serum TGF- $\beta$ 1 levels in subcohorts of healthy Japanese adults were classified by gender, age, and lifestyle factors such as obesity, cigarette smoking and alcohol drinking (Table 1). The results indicate highly significant ( $p < 0.0001$ ) difference in serum TGF- $\beta$ 1 levels between men (mean value:  $37.6 \pm 0.12$  ng/ml, N = 4880) and women (mean value:  $35.1 \pm 0.12$  ng/mL, N = 4254). When men and women were divided into 10-year age groups, men had higher serum TGF- $\beta$ 1 levels in each group compared with women. Serum TGF- $\beta$ 1 levels were inversely associated with age in both men and women, with decreasing serum levels as age advanced (Table 1).

Serum TGF- $\beta$ 1 levels were positively associated with obesity (Table 1). Both men and women whose BMI were 25.0 or more had higher serum TGF- $\beta$ 1 levels compared with those whose BMI were less than 18.5 (trend  $P < 0.0001$ ).

Men who smoked 20 or more cigarettes per day had higher mean serum TGF- $\beta$ 1 levels compared with non-smokers and current smoker who smoked less than 20 cigarettes per day (Table 1). Serum TGF- $\beta$ 1 levels increased with increasing amount of smoking (trend  $P < 0.0001$ ). Serum TGF- $\beta$ 1 levels were also positively associated with alcohol drinking in men (Table 1), with those who drank 23 g or more of alcohol experiencing higher serum TGF- $\beta$ 1 (trend  $P = 0.0008$ ). In women, however, no significant trend was observed in elevated TGF- $\beta$ 1 associated with either smoking (trend  $P = 0.77$ ) or drinking (trend  $P = 0.28$ ).

### 4. Discussion

We examined the association between serum TGF- $\beta$ 1 levels and gender, age, and selected lifestyle factors in a large number of healthy control subjects, and found that serum TGF- $\beta$ 1 levels appear to be modulated in part by gender, age and lifestyle factors such as obesity, cigarette smoking, and alcohol drinking.

In this study, men had higher serum TGF- $\beta$ 1 levels in each group compared with women, and serum TGF- $\beta$ 1 levels decreased with increasing age in both men and women. The inverse association observed with age was consistent with another study of healthy Japanese individuals, which showed an age-dependent decrease with serum TGF- $\beta$ 1 levels being significantly higher in children when compared with adults [14]. However, in previous study by Grainger et al. [10], no significant association was observed between plasma TGF- $\beta$ 1 levels and age in a meta-analysis of 1,006 healthy Europeans subjects. Since this study used the same Quantikine ELISA kit, other factors such as genetic background of the study subjects, different blood compartments, and methods of sample preparation may have contributed to the inconsistent results. The association between serum TGF- $\beta$ 1 levels and aging seems complex because of the dual role of TGF- $\beta$ 1 in human disease. While increased activity occurs in patients with progressive cancers, decreased activity could be observed in early tumorigenesis or atherosclerosis [1]. The age-related decline in serum TGF- $\beta$ 1 levels observed in our study suggests that decreased production of TGF- $\beta$ 1 might be involved in aging process. Since aging is characterized by a decrease in bone volume, there has been

evidence showing that the skeletal content of TGF- $\beta$ 1 and IGF-1 decreased linearly with age (15). This indicates that the growth factor content of the bone could decrease as a function of aging.

We found that serum TGF- $\beta$ 1 levels increased with increasing BMI in both men and women. Several lines of evidence were available to support a positive association between TGF- $\beta$ 1 and obesity. Porreca et al. reported that TGF- $\beta$ 1 levels were significantly elevated in hypertensive obese patients compared with hypertensive patients with normal BMI [16]. In adipose tissue from both obese mice and humans, the levels of tissue TGF- $\beta$ 1 antigen have been shown to correlate well with BMI [17]. Moreover, TGF- $\beta$ 1 gene was associated with obesity phenotypes such as BMI, fat mass, and lean mass in a large sample of white population [18]. Likewise, an association between TGF- $\beta$ 1 polymorphism and both BMI and abdominal obesity was observed in Swedish men [19].

Until now information regarding the effect of smoking or drinking on serum TGF- $\beta$ 1 levels was not available. We found that high amount of tobacco consumption was associated with elevated serum TGF- $\beta$ 1 levels among men. One possible explanation for this positive association relates to the immunosuppressive effect of TGF- $\beta$ 1 on immune system. Chronic inhalation of cigarette smoke alters a wide range of immunological functions, including both innate and adaptive immune responses [20]. Smokers develop airway inflammation through oxidative stress with the involvement of immune cells including macrophages, lymphocytes, and natural killer (NK)-cells [20]. It has been shown that smokers had significantly lower NK cell activity compared with nonsmokers [21], and high natural cytotoxic activity of peripheral-blood lymphocytes has been associated with a decreased cancer risk in a prospective cohort study [22]. In response to cigarette smoke lung epithelium may increase the release of TGF- $\beta$ 1 to regulate those inflammatory cells. The mRNA levels have been shown to be significantly higher in small airway epithelium in smokers when compared with nonsmokers [23].

The strengths of our study include a large sample size, sex-specific analysis, a detailed collection of lifestyle factors such as smoking and drinking, and the ability to adjust for other confounding factors. Our study also has limitations. A causal effect between lifestyle factors and serum TGF- $\beta$ 1 cannot be proved with our cross-sectional analysis. A longitudinal analysis would be more suitable to examine long term influences, but few such studies have been reported. Another

limitation is that we only measured serum TGF- $\beta$ 1 at baseline. Using one point measurement might have failed to catch the variations in TGF- $\beta$ 1 levels, given the dual role that TGF- $\beta$ 1 plays in disease process.

In conclusion, serum TGF- $\beta$ 1 levels appear to be modulated by gender, age and lifestyle factors such as obesity, cigarette smoking, and alcohol drinking in healthy Japanese adults.

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